

through 1) the similar antibody used in the G1/G2 ELISA assay, F-78, binding to a repetitive epitope exposed twice on G1 and G2 or, 2) by BC-3 detecting the aggrecanase-generated N-terminal <sup>374</sup>ARGSVI sequence.

**Results:** Induction of cartilage degradation *ex vivo*, resulted in 10 fold elevated aggrecanase fragments (<sup>374</sup>ARGSVI-G2) ( $P=0.02$ ) and 9 fold increased total aggrecan release (G1/G2) ( $P=0.02$ ), compared to that of vehicle control. Total aggrecan levels in RA patients were significantly decreased from  $824.8 \pm 31$  ng/ml in healthy controls to  $570.5 \pm 30$  ng/ml, corresponding to an approximate 31% decrease ( $P<0.0001$ ). Western blot analysis for total aggrecan showed one strong band detected at 10 kDa, and weaker bands at 25 and 45 kDa in both healthy controls and RA patients. With regards to aggrecanase activity, only one strong band in RA patients of 45 kDa was detected.

**Conclusions:** This study is the first to characterize different aggrecan fragments in human serum. We clearly demonstrate that total aggrecan levels are lowered in RA patients. In alignment, RA patients had highly elevated aggrecanase-mediated aggrecan degradation, providing a rationale for the decreased aggrecan levels. These data may provide a new diagnostic tool for monitoring joint-destruction in RA patients, and allow for early assessment of response to treatment prior to that of traditional radiographic techniques.

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### CHARACTERIZATION OF METALLOPROTEINASE CLEAVAGE PRODUCTS OF HUMAN ARTICULAR CARTILAGE

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**Purpose:** The present study measures the effects of metalloproteases on human articular cartilage. Supernatants from digestions with these enzymes were analyzed via proteomics methods to characterize post-translational modifications of cartilage peptides, to determine specific cartilage protein cleavage sites of different metalloproteases to better understand their selectivities, and to measure release of peptides that may serve as useful biomarkers of cartilage degradation. The metalloproteases utilized in this study have been reported to be overexpressed and active in arthritic cartilage, so determination of their substrates and products may lead to a better understanding of their potential role in disease and to identification of new targets and biomarkers of cartilage degradation.

**Methods:** Human articular cartilage was digested by addition of exogenous metalloproteases, including MMP-2, -3, -8, -9, -12, -13, ADAMTS-4, and ADAMTS-5, and proteolyzed peptide products were identified by proteomics methods using mass spectrometry.

**Results:** Complete sequences of peptides, including N- and C-termini and post-translational modifications were determined. Abundant peptides originating from collagen types I, II, and III, biglycan, prolargin, fibromodulin, fibronectin, decorin, cartilage oligomeric matrix protein, cartilage intermediate layer protein, megakaryocyte stimulating factor, mimecan, aggrecan, and lumican were identified after metalloprotease digestion. Specific collagen type II peptide biomarkers, including those containing the 3/4 – 1/4 cleavage site and those containing the Helix-II and CTX-II domains, were observed after release by selected proteases.

**Conclusions:** this study identifies many of the most abundant metalloprotease cleavage products of human articular cartilage. The list of peptides generated by these digestions provides insights into the broad number of substrates susceptible to specific

metalloprotease proteolysis in diseased cartilage. Many of these peptides are potential biomarkers of arthritis or of metalloprotease activity in articular cartilage. Further evaluation of many of these peptides as biomarkers might be achieved by quantitation in synovial fluid, blood, or urine.

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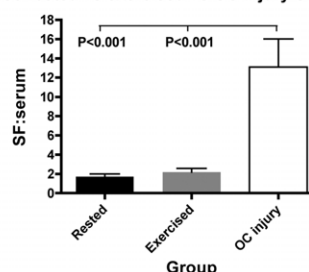
### EFFECTS OF EXERCISE AND OSTEOCHONDRAL INJURY ON CTX II CONCENTRATIONS IN EQUINE SYNOVIAL FLUID AND SERUM

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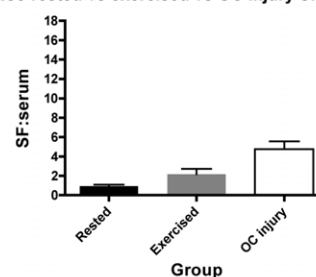
**Purpose:** Collagen type II is the predominant collagen in articular cartilage and is highly specific for this tissue. A biomarker of crosslinked C-telopeptide fragments of type II collagen (CTX II) has been used to assess severity and progression of cartilage degradation in humans. The objectives of the current study were to evaluate the effects of exercise and osteochondral (OC) injury on concentrations of CTX II fragments in synovial fluid (SF) and serum of horses.

**Methods:** SF and serum samples were taken from 3 groups of Thoroughbred racehorses: (1) rested horses ( $n=40$ ), (2) exercised horses: group 1 horses had the same joints sampled after 4-7 months of race training ( $n=40$ ) and (3) OC injured horses: racehorses that had arthroscopic surgery for removal of OC fragments resulting from racing injury ( $n=44$ ). From group 1 and 2 horses, SF was obtained from 20 metacarpophalangeal (MCP),

#### A MCJ rested vs exercised vs OC injury SF:serum



#### B RCJ rested vs exercised vs OC injury SF:serum



#### C MCP/MTP rested vs exercised vs OC injury SF:serum

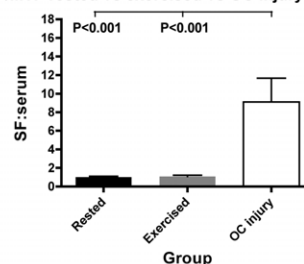


Figure 1. Comparison of SF to serum CTX II concentrations (SF:serum) in rested, exercised, and OC injured horses. A. MCJ - middle carpal joint; B. RCJ - radiocarpal joint; C. MCP/MTP - metacarpophalangeal/metatarsophalangeal joint.

10 middle carpal (MCJ), and 10 radiocarpal (RCJ) joints (n=40). SF samples from group 3 horses were from 16 MCP, 6 metatarsophalangeal (MTP), 12 MCJ, and 10 RCJ (n= 44). Serum CTX II was measured for all horses (n=124). SF and serum CTX II concentrations were measured by an ELISA (Pre-Clinical CartiLaps®) previously validated for use in equine serum and SF. Differences between groups in serum and SF CTX II concentrations and the ratio (SF:serum) were evaluated using one-way ANOVA with Tukey's test for multiple pairwise comparisons.  $P < 0.05$  was considered significant.

**Results:** Concentrations of CTX II were significantly elevated in SF from all joints of horses with osteochondral fragmentation compared to rested ( $P < 0.05$ ). Concentrations of CTX II were significantly elevated in SF from MCP/MTP and RCJ of horses with osteochondral fragmentation compared to exercised horses ( $P < 0.05$ ). There was no significant difference in SF CTX II between rested and exercised horses for any of the joints analyzed. Serum CTX II concentrations were significantly lower in horses with OC injury in MCJ and RCJ compared to rested horses ( $P < 0.01$ ). Horses with MCJ OC injury had significantly lower serum CTX II concentrations than exercised horses ( $P < 0.01$ ), but horses with RCJ and MCP/MTP OC injury did not. SF to serum ratios (SF:serum) were significantly higher in horses with MCP/MTP and MCJ OC injury compared to rested and exercised horses ( $P < 0.001$ ) (Fig. 1). A significant difference was found between MCJ and RCJ OC injury SF:serum ( $P < 0.05$ ).

**Conclusions:** These results strongly support the use of SF and serum CTX II concentrations as a discriminating biomarker for the diagnosis of OC injury in equine joints.

**Acknowledgements:** Study supported by the American College of Veterinary Surgeons.

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### COLL2-1 AND COLL2-1 NO<sub>2</sub>: MARKERS OF EARLY DISEASE IN THE HARTLEY GUINEA PIG MODEL OF SPONTANEOUS OA

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**Purpose:** Several different type II collagen epitopes have been described as potential biomarkers of onset and/or progression of OA. Among the degradative markers, the C-telopeptide fragment, CTXII, has the most abundant data supporting its use as an arthritis marker. More recently, two serum immunoassays have been developed to quantify a sequence derived from the triple helical region of type II collagen, (Coll2-1) and its nitrated form (Coll2-1 NO<sub>2</sub>), that can be used to quantify both cartilage degradation as well as oxidative damage, respectively. In this study, we investigated the utility of serum Coll2-1, Coll2-1 NO<sub>2</sub> and CTXII in a well characterized cohort of Hartley guinea pigs.

**Methods:** Forty six male Hartley guinea pigs were sacrificed at 3 weeks, 2, 4, 7, 10, 12 and 18 months of age, at which time blood samples were obtained. Histological severity of OA was determined using a semi-quantitative grading scheme described previously. Serum Coll2-1 and Coll2-1 NO<sub>2</sub> were quantified by competitive ELISA and serum CTXII were quantified using the Serum Pre-clinical Cartilaps ELISA. This assay was designed to detect degradation products of C-terminal telopeptides of type II collagen in animal sera. Statistical analyses included: the non-parametric Mann Whitney test to compare the levels of Coll2-1 at 3 weeks and 4 months of age, and correlations between Coll2-1 and total histological score estimated by the non-parametric Spearman's rank correlation coefficient. Data were considered statistically significant at  $p$  value  $< 0.05$ .

**Results:** CTXII and Coll2-1 displayed completely different time course profiles (Fig. 1). At 3 weeks of age, CTXII was elevated

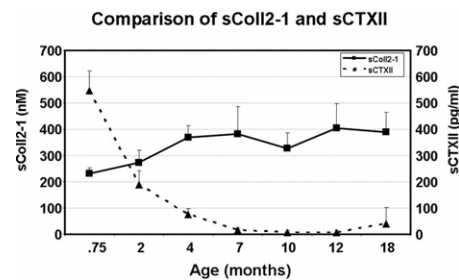


Figure 1

and rapidly declined to very low levels by 7 months of age, showing very little change from 7-18 months of age. In contrast, Coll2-1 displayed a nadir at 3 weeks, a 65% increase from 3 weeks to 4 months of age ( $p = 0.002$ ) with consistently elevated levels thereafter to 18 months of age. The serum profile for Coll2-1NO<sub>2</sub> was similar but concentrations equaled 1-2% the serum concentrations of Coll2-1 throughout the time course. The early change in Coll2-1 correlated with an increase in OA histological severity during this time interval ( $r = 0.75$ ,  $p = 0.0004$ ). Mean serum Coll2-1 was significantly different between the lowest quartile of OA histological severity and the higher three quartiles (Fig. 2).

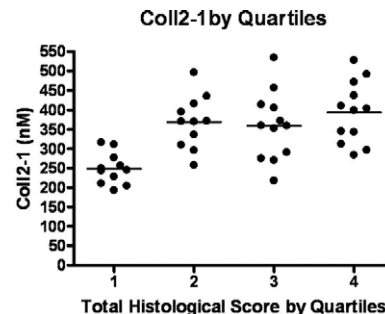


Figure 2

**Conclusions:** Although CTXII and Coll2-1 are both type II collagen specific epitopes, it is clear that they reflect different biological processes, probably as a result of differential expression of collagen degradative enzymes in different joint tissue compartments. For instance, the serum CTXII profile in the guinea pig is most compatible with collagen II turnover in the growth plate cartilage which ceases at 4 months of age in this model and is barely measurable during the period of development of histological OA. In contrast, the levels of Coll2-1 and Coll2-1 NO<sub>2</sub> are lowest during the most active period of growth plate cartilage turnover, increase markedly in young animals when collagen birefringence data suggests collagen disruption is occurring, and remain elevated during the course of disease development. These results suggest that the Coll2-1 and Coll2-1NO<sub>2</sub> epitopes are likely generated from articular cartilage and may be useful as quantitative biomarker outcomes for early disease prevention and treatment in this model system.

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### BIOMARKERS ASSOCIATED WITH CLINICAL PHENOTYPES OF HAND OSTEOARTHRITIS IN A LARGE MULTIGENERATIONAL FAMILY: GIAFD FAMILY

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**Purpose:** The goal of this study was to evaluate the relationship between biological markers and osteoarthritis (OA) clinical phenotypes in a large multigenerational family in the United